Poly-L-Lysine/Heparin Multilayer Coatings Prevent Blood Protein Adsorption

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Abstract

The adsorption of blood proteins, serum albumin (BSA), immunoglobulin G (IgG) and fibrinogen (FGN), onto model SiO_2 planar surfaces coated with poly-L-lysine/heparin multilayers (PLL/HEP) has been investigated by means of ellipsometry and quartz crystal microbalance with dissipation. Aiming at the development of low fouling coatings, this study has been focused on the effects that the number of layers and the type of polyelectrolyte present on the topmost layer have on the adsorption of these proteins. The three proteins interact with PLL-ended coatings whereas HEP-ended coatings prevent the adsorption of both BSA and IgG and induce a decrease in the adsorbed amount of FGN, down to 0.4 mg/m^2 for three bilayers, as the number of PLL/HEP bilayers increases. These results suggest that heparin-ended multilayers prevent protein adsorption, which is an indicative of good blood compatibility. As a consequence we propose that PLL/HEP coatings could be used for the development of vascular medical devices.

Keywords: Layer by layer; protein adsorption; ellipsometry; QCM-D; heparin coating

1. Introduction

Protein adsorption has become a major problem for the successful performance of biomaterials. As soon as a foreign material gets in contact with blood the proteins present in the fluid will adsorb at the interface either in a reversible or in an irreversible manner depending on the nature of both the material and the protein [1]. Because some of these proteins, like immunoglobulin G (IgG) and fibrinogen (FGN), are involved in the activation of the complement system or in the clotting cascade [2, 3], fouling may lead to the degradation of the material [4] or clot formation onto it [3].

Many efforts have been made control protein adsorption onto surfaces, especially by coating the surfaces by means of self-assembled monolayers (SAMs) [5-7] and, in recent years, polyelectrolyte multilayers (PEMs) [8, 9]. The purpose of most of these strategies is to change the physicochemical properties of the surface rendering either a low fouling interface or a selective one that only binds specific biomolecules. PEMs fabricated by the layer by layer method have become one of the most popular ways to modify planar surfaces [8-15] and colloidal particles [16, 17] providing them with tailor made properties such as controlled thickness, roughness, wettability and rheology. This method consists in alternatively depositing either natural or synthetic polymers onto a surface by exposing it to solutions that contain the polymer of interest. The interaction forces that drive the build-up of the multilayer are mainly electrostatic [10, 11, 13, 15, 18] however, coatings assembled through hydrophobic forces [12] and hydrogen bonds have also been developed [19]. The popularity of PEMs does not come only from its simplicity but also from the wide range of applications they have. Apart from antifouling coatings [8, 9], they have been extensively used in drug delivery [17] and biosensing devices [14, 20].

We have previously studied the effects that pH and the underlying substrate have on the build-up of poly-L-lysine/heparin (PLL/HEP) multilayers [11]. Preventing protein fouling onto the surface of vascular medical devices is essential for their proper operation. Therefore, in the present study, as a step forward towards their application as hemocompatible coatings for biomedical devices and with the aim of developing low fouling coatings, we have investigated what are the effects on protein adsorption of both the polymer present on the outermost layer of the coating as well as the number of layers. For this purpose we have chosen three relevant blood proteins such as serum albumin, which is the most abundant protein present in blood, and immunoglobulin G and fibrinogen known for their central role in the activation of complement system and the clotting cascade [2].

2. Materials and methods

2.1 *Chemicals*

All chemicals were purchased from Sigma-Aldrich. Poly-L-lysine hydrobromide (MW 30000-70000 g/mol), heparin sodium salt from intestinal mucosa, bovine serum albumin, human IgG and human fibrinogen were also purchased from Sigma-Aldrich and used without further purification. All solutions were prepared with Mili-Q water (18.2 MΩ▪cm, Millipore, Billerica, MA, US)

2.2 *Surfaces*

Ellipsometry experiments were performed on silicon wafers with a silica layer of approximately 300 Å (Semiconductor Wafer Inc. Hsinchu, Taiwan). Hydrophilic silica substrates were cleaned following the procedure developed at RCA laboratories [21]. The silica substrates were boiled for 5 minutes in an alkaline solution; rinsed extensively with water; boiled again for 5 minutes in an acidic solution; and finally rinsed with water and ethanol. The components of the alkaline solution were: NH_4OH (25%), H_2O_2 (30%), and water with a volume proportion of 1:1:5 respectively. The components of the acidic solution were: HCl (37%), H_2O_2 (30%), and water with a volume proportion of 1:1:5 respectively. At the end of the cleaning procedure the surfaces were stored in ethanol. Prior to use, the surfaces were plasma cleaned for 5 minutes in low pressure residual air using a glow discharge unit (PDC-32 G, Harrick Scientific Corp., USA).

QCM-D measurements were performed on AT-cut 5 MHz quartz crystals (Q-Sense E4, Biolin Scientific AB, Sweden) and had silica as outermost layers. The substrates were cleaned according to the instructions from the manufacturer: 1) 10 minutes plasma treatment; 2) 30 minutes immersion into 2% SDS solution 3) extensive rinsing with water; 4) 10 minutes plasma treatment. Both cleaning procedures yielded hydrophilic surfaces with water contact angles less than 10° as measured with a drop shape analyzer, (DSA100, Krüss GmbH, Hamburg, Germany).

2.3 *Multilayer build-up and protein adsorption studies*

All measurements were done in Dulbecco's phosphate buffered saline (ionic strength, I = 0.148 M), pH 7.4 (DPBS) at 25 °C and concentrations of 0.02 mg/ml and 0.2 mg/ml for the polyelectrolytes and protein adsorption experiments respectively. The adsorption of PLL and HEP was monitored in solution for 5 minutes with a 5 minutes long rinsing step with polyelectrolyte-free buffer solution between each polyelectrolyte addition. After the last polyelectrolyte layer was formed and rinsed the protein solution was supplied and its

interaction with the coated surface was monitored for 30 minutes. In order to elucidate whether protein adsorption was reversible or irreversible, a final 30 minutes rinsing step with DPBS was done.

The instruments employed were a Rudolph thin film ellipsometer (type 43603-200E, Rudolph Research, USA) automated according to the concept of Landgren and Jönsson [22] with an experimental setup based on null ellipsometry according to the principles of Cuypers [23]. A xenon arc lamp was used as the light source, and light was detected at 442.9 nm using an interference filter with UV and infrared blocking (Melles Griot, The Netherlands). The trapezoid cuvette made of optical glass (Hellma, Germany) was equipped with a magnetic stirrer (325 rpm). The adsorbed amount, Γ, was calculated by using de Feijter's equation [24] (Eq. 1), where n_f is the refractive index and d the thickness of the mixed polyelectrolyte multilayer. Although the *dn/dc* value for heparin is 0.13 ml/g [25], the value for PLL, *dn/dc* = 0.15 ml/g [26], has been used for the whole multilayer. This procedure will introduce an underestimation for the adsorbed amount of heparin [27].

$$\Gamma = d\,\frac{n_f - n_{Buffer}}{dn/dc} \quad (1)$$

Proteins adsorbed amounts were estimated in the same way as the ones obtained for the PEMs considering the PEM-protein complex as a single layer, and by using a refractive index increment value of 0.18 ml/g [24]. The adsorbed amount of the protein layers was then obtained by applying the following expression: $\Gamma_{PEM+Prot}(0.18) - \Gamma_{PEM}(0.18)$ (the same procedure was applied to the thickness, d_{Ellips}) under the assumption that protein adsorption did not induce any changes in the optical properties of the multilayers.

The QCM-D measurements were performed by using a (Q-Sense E4 system, Biolin Scientific AB, Sweden). Both polyelectrolyte and protein solutions were supplied by means of a peristaltic pump at a flow rate of 0.1 ml/min. A detailed description of the technique and its basic principles can be found elsewhere [28]. Briefly, an alternating-current voltage is applied through a gold-coated quartz chip to stimulate the shear mode oscillation of the quartz crystal. When a certain amount of mass is adsorbed onto the sensor chip, a proportional decrease in the resonance frequency, Δf, will be detected as stated in equation 3, known as Sauerbrey's equation Sauerbrey [29]:

$$\Delta f = -\frac{n\Delta m_s}{C} \quad (3)$$

where n is the overtone number (n= 1, 3, 5 …), C is the mass-sensitivity constant (C = 0.177 mg/m^2), and the subscript 's' stands for Sauerbrey. From this relation, a rough estimate of the

mass can be made when the film deposited onto the chip can be considered rigid. Therefore, Sauerbrey's equation was used to determine the PEMs as well as the BSA and IgG adsorbed amounts. However, due to the high dissipation values obtained for the FGN films and the complexity of the system it was not possible to determine the mass with this technique. A qualitative analysis of the viscoelasticity of the PEM coatings and proteinaceous films was done by plotting ΔD *vs* Δf and analyzing the changes in the slope [11, 30].

Coating the QCM-D sensors with the PEMs rendered very hydrophilic surfaces (water contact angle, WCA < 10°) according to the measurements performed with a drop shape analyzer (OCA Plus 20, Dataphysics Instruments GmbH, Filderstadt, Germany).

An atomic force microscope (AFM) (Asylum MFP-3D-SA Santa Barbara, USA) was used to analyze the topography of the coated QCM-D sensors using AC240TS (Olympus) cantilevers in tapping mode. The probes were calibrated to 1V of free air using the GetReal™ function of the Asylum Research scanning software (V13, Asylum, Santa Barbara, USA). Scans were 20µm x 20µm in size with a resolution of 512 x 512 pixels. The obtained images were further optimized and analyzed with Gwyddion (V2.43, Department of Nanometrology, Czech Metrology Institute, Brno, Czech Republic). Image optimization included plane leveling and removing line artefacts.

2.4 *Statistics*

All ellipsometry experiments were performed twice and the results are presented as mean values ± deviation from the mean while the QCM-D measurement were run in triplicate and the results are presented as the mean values ± standard deviation.

3. Results and discussion

3.1 *Build-up and characterization of PLL/HEP multilayers*

The build-up of the multilayer film was followed in situ by means of null-ellipsometry and QCM-D as it is shown in Fig. 1. As SiO_2 surfaces are negatively charged under our experimental conditions the first layer was formed by supplying a poly-L-lysine (PLL) solution, poly-L-lysine is positively charged at the working pH (isoelectric point ~ 10.5 [31]) and has a zeta potential of ≥ 20 mV at our experimental conditions [31]. After a rinsing step with polyelectrolyte free buffer, the heparin (HEP) solution was added, with HEP negatively charged due to the low pK_as of the chemical groups that form the molecule: $pK_a^{Carboxyl} \sim 3$ and $pKa^{Sulfate} < 2$ [32], and the cycle was repeated as many times as needed in order to obtain the desired number of layers. As it can be seen (Fig.1), by alternatively supplying oppositely charged polyelectrolyte solutions produces an increase in the ellipsometric mass and a

decrease in the frequency measured by QCM-D. This shows that alternate layers composed of PLL and heparin (HEP) are being formed on the surface. According to Picart et al. the alternate deposition of oppositely charged polyelectrolytes, PLL and hyaluronic acid (HA), leads to a surface charge reversal from positive to negative values [15]. Since PLL/HEP and PLL/HA multilayers both show an exponential growth and both HEP and HA are polysaccharides we can assume an analogous behavior for both [33] and therefore that the charge reversal effect will also take place in our system.

Polyelectrolyte multilayers (PEMs) can be grouped into two main categories depending on the adsorbed amount (mass) behavior with respect to the layer number, linear or exponential [13, 34]. In the present study, by following the procedure proposed by Bieker and Schönhoff it was found out that PLL/HEP films show an exponential behavior for Γ, suggesting the diffusion of at least one of the components of the film [13, 15, 34], in good agreement with previous studies where PLL was one of the components of the film [11, 13, 15].

A more detailed analysis of the build-up process can be done by analyzing the individual contribution of each polyelectrolyte layer (SM1) where QCM-D results show three different growth regimes. The first regime would correspond to the formation of the first couple of PLL/HEP bilayers, $(PLL/HEP)_2$, and would be strongly influenced by the SiO_2 surface and the adsorption of the initial PLL monolayer. In this regime, the contribution of PLL to Γ_{QCM} increases once heparin is present onto the surface, indicating that the coating becomes more hydrated as it grows away from the surface, an interpretation that is supported by the water content calculations (Figure 1C). During the second regime the contribution of PLL to Γ_{QCM} slightly decreases with the number of layers while the contribution of HEP increases linearly suggesting that HEP-ended coatings are more hydrated than PLL-ended ones. In the third regime the contribution of both PLL and HEP increases with the number of layers where the increments produced by HEP are being larger compared to those for PLL. Picart et al. proposed that these regimes produced by the initial formation of PEM islands that ended up merging for a certain amount of layers [15]. However, from the AFM images that we obtained which indicate a full coverage of the surface (Figure 2) we would rather attribute the regimes to the to the vanishing influence of the substrate, the subsequent formation of different types of PLL/HEP complexes [11] and the changes in topography between PLL-ended and HEP-ended coatings as measured by means AFM (Figure 2). All these results, together with the fact that for Γ_{Ellips} the highest contribution is for PLL throughout the whole process, would

also indicate that the incorporation of HEP increases the hydration level of the coatings making them softer (more dissipative).

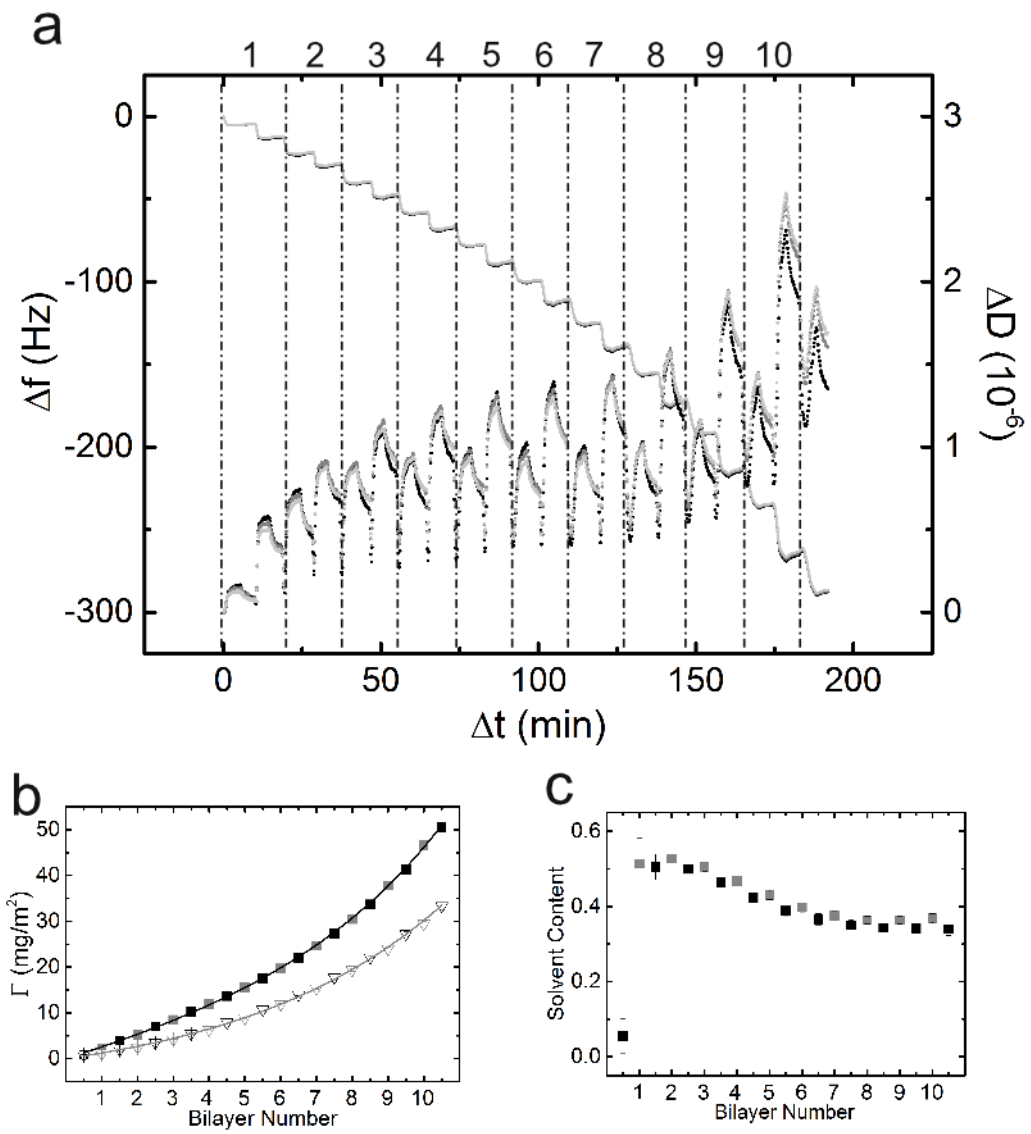

Figure 1. Ellipsometry and QCM-D Characterization of PLL/HEP multilayers. (a) Changes in frequency and dissipation produced by the consecutive adsorption of PLL and HEP onto the SiO_2 surface for the 3rd, 5th and 7th overtones (Black, grey and light grey respectively) as measured by QCM-D. (b) Increase in the adsorbed amount, Γ, produced by the build-up of the multilayer measured by means of ellipsometry (trianlges) and QCM-D (squares). The lines correspond to the best fit obtained for the equation $y(x) = Ax + B[\exp(cx) - 1]$[34]. (c) Solvent content of the multilayers calculated from the difference between the $\Gamma_{\text{QCM-D}}$ (Sauerbrey) and Γ_{ellips}. In (b) and (c) black symbols correspond to PLL and grey symbols to HEP. The formation of the film was performed in DPBS buffer pH 7.4 at 25 °C for 0.02 mg/ml polyelectrolyte solutions.

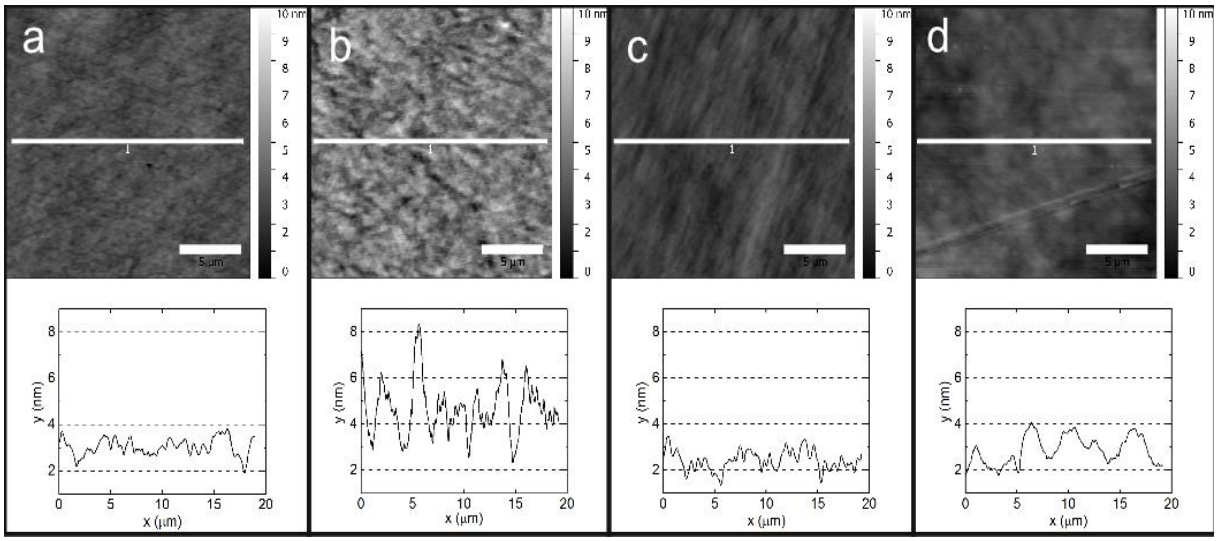

Figure 2.AFM images and topography profiles of the SiO_2 coated quartz used for the QCM-D measurements. SiO_2 surfaces were coated with (a) PLL, (b) $(PLL\text{-}HEP)_1$ bilayer, (c) $(PLL\text{-}HEP)_2$-PLL, and (d) $(PLL\text{-}HEP)_3$. Measurements were performed in DPBS buffer pH 7.4 at room temperature. Scan areas are 20 x 20 μm^2 and the scale bars correspond to 5 µm length.

3.2 *Protein adsorption*

In order to determine the ability of the PLL/HEP coatings to prevent blood protein fouling we have studied what is the effect of both the layer number and the polyelectrolyte on the topmost layer on the adsorption of three relevant blood proteins: serum albumin, immunoglobulin G and fibrinogen. Each of them shows a different behavior depending on the surface the protein solution is exposed to and throughout the following sections we will present and discuss the results obtained.

3.2.1Bovine serum albumin.

BSA, which is negatively charged at our experimental conditions (isoelectric point ~ 5.1 [35]), is only able to adsorb onto the positively charged PLL-ended coatings (Figure 3). Furthermore, a deeper analysis of ellipsometric results indicates that both the adsorbed mass and the adsorption rate (SM2) are unaffected by the number of layers. The 3-D structure of BSA can be approximated to an equilateral triangle with 8 nm side and 3 nm depth [36] with a hydrodynamic radius, R_H, of ~ 4 nm [35]. From these values the theoretical adsorbed amount for a BSA monolayer can be determined depending on the orientation the proteins adopt on the surface. In Table SM2-1 these values are compared with ellipsometric adsorbed amount, Γ_{Ellips}. There it can be noticed that the measured values are below the theoretical ones, Γ_{Ellips} ~1/2 $\Gamma_{Th}^{Side\text{-}On}$. This is in good agreement with previous calculations based on the RSA model were it was demonstrated that for spherical particles adsorption at planar interfaces the maximum coverage is ~ 55% [37].

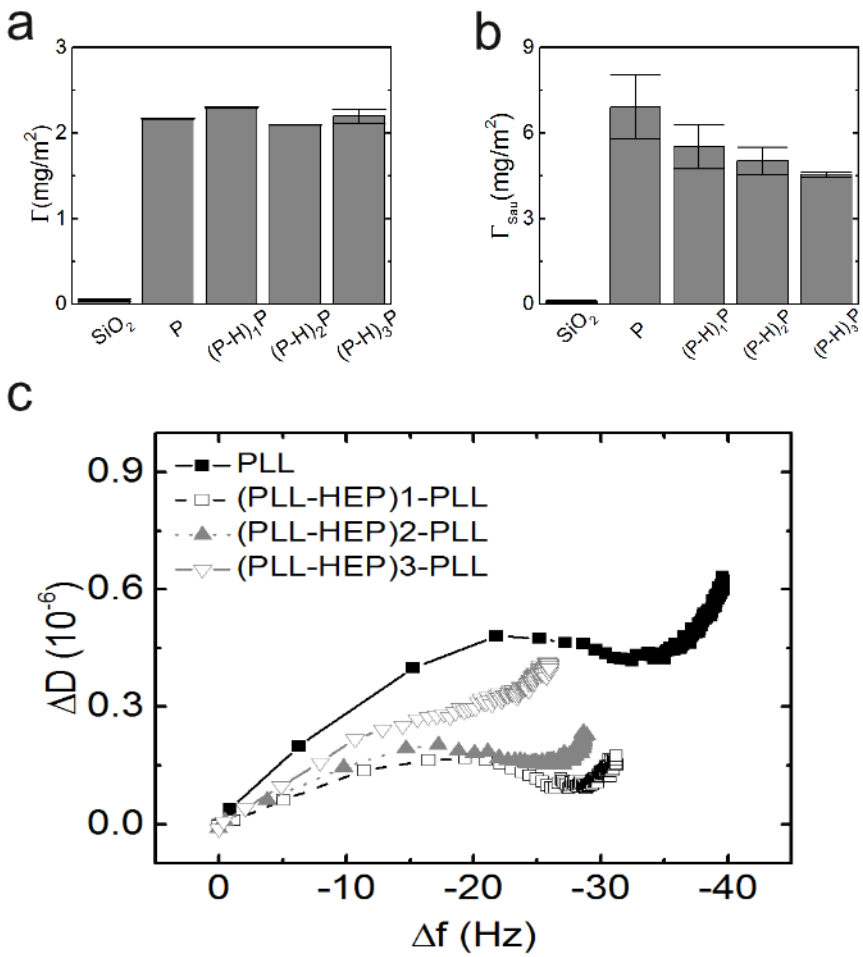

Figure 3. BSA onto PLL-ended coatings. (a) Ellipsometric and (b) QCM-D adsorbed amount after 30 minutes of adsorption. (c) ΔD vs Δf plots for the 30 minutes adsorption. Experiments run at 25 °C and pH 7.4 for a protein concentration of 0.2 mg/ml in DPBS

The hypothesis of conformationally changed proteins in the layer can be supported by analyzing Fig. 3c. The adsorption of BSA onto PLL can be divided into 3 different regions with different slopes. The first region (5 Hz < Δf < 15 Hz depending on the number of PLL layers) would represent a fast adsorption in a native state, most probably orthogonally to the surface [38], that is followed by a conformational change of the adsorbed proteins in order to maximize their contact area, decrease in $|\Delta D/\Delta f|$. These proteins will form an irreversibly bound layer as has been previously described [39]. The third region, where the slope is highest, indicates that proteins in solution keep on binding to the surface in a loosely attached configuration. The small contact area with the surface together with the electrostatic repulsion between the negatively charged BSA molecules would result in a reversible adsorption and their subsequent desorption during the rinsing step SM2.

It is worth mentioning that all the dissipation values measured with QCM-D for the BSA films are below $1x10^{-6}$ indicating that they are very rigid and also that the differences in ΔD

observed for the adsorption onto the different layer numbers are not significant [39]. Due to the rigidity of the BSA layers, we have used the Sauerbrey equation to calculate the hydrated mass, Γ_{QCM}, and thus estimate the solvent content of these films (Figure SM3).

The fact that bare SiO_2 surfaces and HEP-ended multilayers prevent BSA from adsorbing can be easily explained by their hydrophilicity and negative charge that would exert a strong electrostatic repulsion [40].

3.2.2 Human immunoglobulin G.

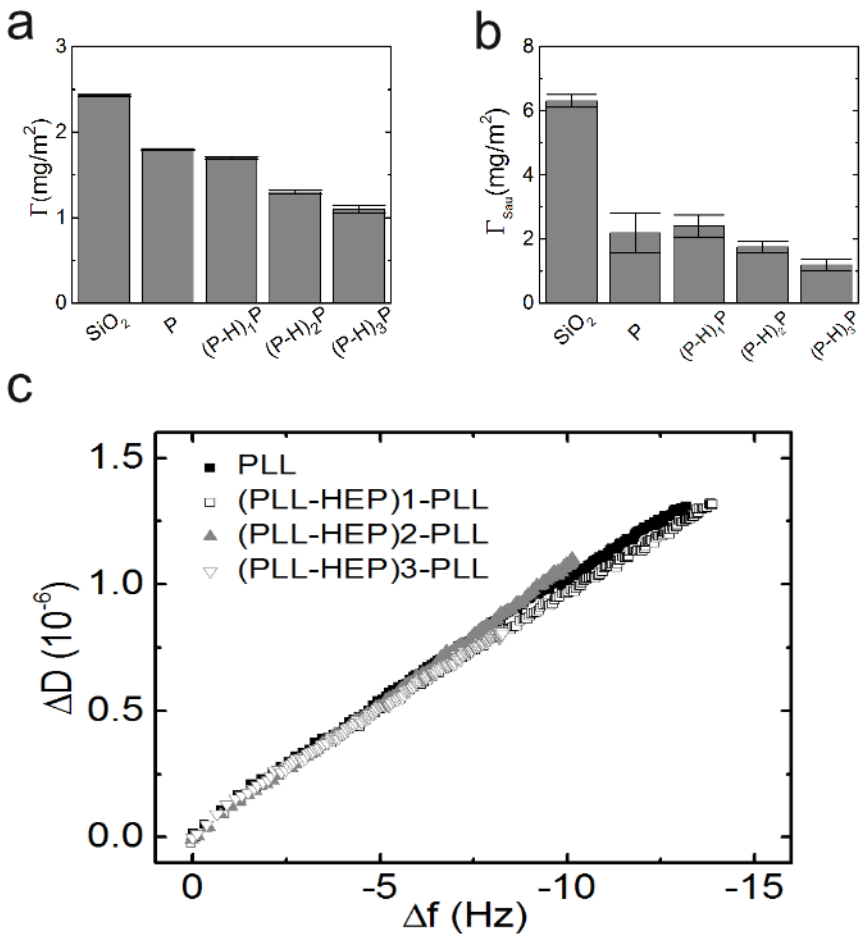

Figure 4. IgG onto PLL-ended coatings. (a) Ellipsometric and (b) QCM-D adsorbed amount after 30 minutes of adsorption. (c) ΔD vs Δf plots for the 30 minutes adsorption. Experiments run at 25 °C and pH 7.4 for a protein concentration of 0.2 mg/ml in DPBS

IgGs have been described to be formed by two main structural units, two Fab units and a Fc region that adopt a 'Y' 3D conformation with approximately 14 nm length, 10 nm width and 5 nm thick [41, 42] having a R_H = 5.5 nm and an isoelectric point ~ 5.8 [43]. Γ_{Ellips} obtained for the IgG layers formed onto the positively charged PLL-ended surfaces were lower than the ones obtained for the negatively charged SiO_2 and decreased with increasing number of PLL

layers (Figure 4) yielding statistical different surface coverages. This was also manifested in the adsorption rates (SM2-2). These results, together with ellipsometric thickness, d_{Ellips} (SM2-3), suggest that, when it adsorbs onto SiO_2, IgG adopts a mixture of end-on (or head-on) and side-on configurations while for PLL-ended coatings the side-on configuration becomes increasingly predominant as the number of layers increases. This hypothesis is supported by the values presented in Table SM2-1 and by higher water content of the IgG layer obtained for the adsorption onto SiO_2 (SM3), ~ 25% for PLL-ended coatings and ~ 60% for SiO_2, which could be indicating a rougher layer resulting from the combination of both configurations. ΔD vs Δf plots also show differences between the adsorption onto SiO_2 and PLL-ended multilayers. While in the latter case the curves show a linear relation between ΔD and Δf, indicative of a monophasic process, for silica the curve has a biphasic character suggesting that either a reorganization of the protein layer is taking place or that proteins adopt a different configuration as the surface gets saturated [44].

3.2.3 Human fibrinogen.

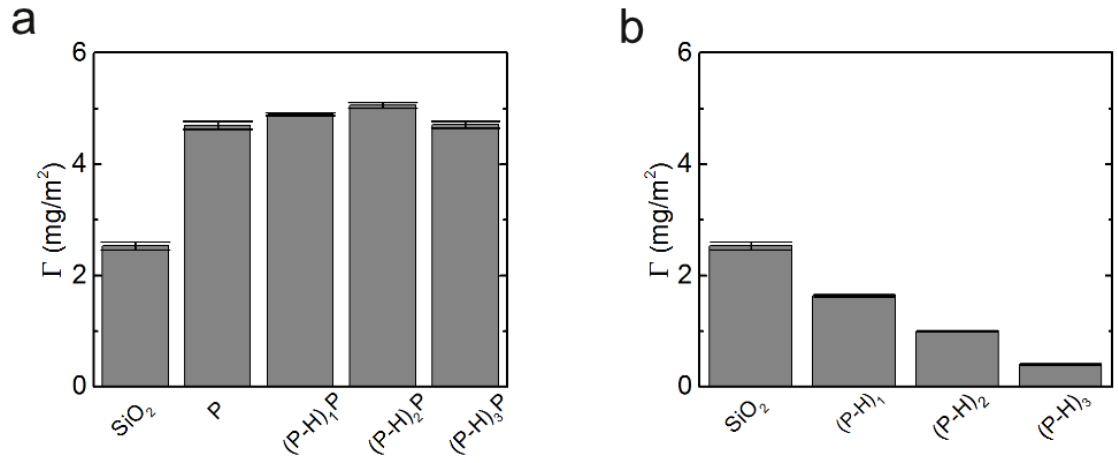

Figure 5. Interaction of FGN with SiO_2 surfaces coated with PLL/HEP multilayers measured by means of ellipsometry. The panels represent the adsorbed amount, Γ, after 30 minutes of adsorption onto PLL-ended coatings (a) and onto HEP-ended coatings (b). Experiments run at 25 °C and pH 7.4 for a protein concentration of 0.2 mg/ml in DPBS.

Fibrinogen is the biggest protein among the ones that we have used in this study with an approximate molecular weight of 340 kDa and R_H = 12.8 nm [45]. It is formed by three covalently linked polypeptide chains Aα, Bβ and γ [46] and it adopts different conformations depending on the environmental conditions [47]. According to the model based on the theoretical calculations made by Adamczyk and coworkers the protein has a heterogeneous charge distribution with the core part exhibiting the highest negative charge while the positive charge is located at the periphery, in the extended Aα chain ends [47]. The heterogeneity in

the charge distribution would explain why, even though FGN is negatively charged under our experimental conditions (IP = 5.8) [45], it is able to adsorb onto both the positively PLL surfaces and the negative SiO_2 substrate (Figures 5 & 6), in good agreement with previous studies [48-50]. However, the different surface coverages obtained in the two cases indicate different conformations of the protein, as can be deduced from the ΔD vs Δf plots as well (Figure 6). These plots indicate clear structural differences in the adsorption process between both types of surfaces. For silica (SM4) a behavior resembling the one observed for BSA suggests that the protein changes its conformation/unfolds upon contact with the surface [49]. For PLL-ended coatings the linear relation between ΔD and Δf throughout most of the adsorption indicates that PLL coatings induce no structural changes on FGN. Furthermore, the increase in the slope at the end of the curves suggests a more loosely bound and dissipative conformation at the end of the adsorption process. This could be due to the adsorption in an end-on conformation or to the formation of a protein multilayer. According to Zeliszewska et al. the former explanation is to be discarded due to electrostatic repulsion effects [50]. The maximum surface coverage for FGN side-on adsorption has been estimated to be ~ 29% of the total surface coverage [51] that compared to our results and those obtained by others [48] for the adsorption of FGN onto PLL suggest that the formation of a protein multilayer is the most plausible explanation. The elutability of the FGN films (Figure SM2-1and table SM2-2) would also support this hypothesis.

On the other hand, when heparin is on the outermost layer of the coatings a different behavior is observed for FGN adsorption. As in the case of IgG adsorbing onto PLL-ended multilayers, Γ decreased as the number of layers increased (Figure 5B) down to 0.4 mg/m^2, very close to the values obtained for PEG-coated surfaces [48]. However, rather than indicating differences in the conformation of the adsorbed protein, this decrease in Γ is produced by the lower adsorption of FGN onto HEP-ended coatings. Several studies have shown that heparin/heparin-like modified surfaces reduce FGN adsorption [18, 52, 53] and the effect was attributed to the high hydrophilicity of the coating and the electrostatic and steric repulsion exerted by the highly sulfated molecule. The decrease in mass observed for the increasing number of layers could be an effect of the higher concentration of HEP on the surface as a result of the exponential growth of the multilayer as has been discussed in the section 3.1 *Build-up and characterization of PLL/HEP multilayers*.

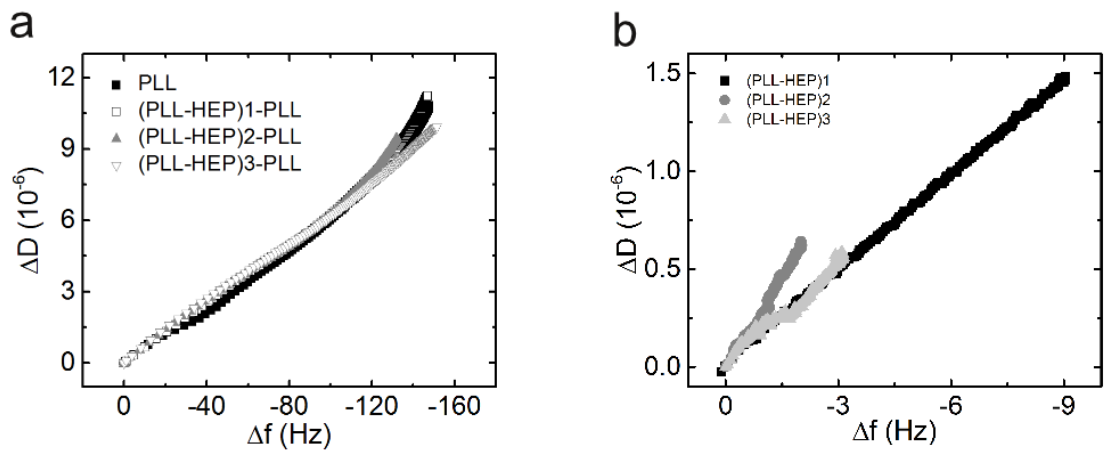

Figure 6. ΔD vs Δf plots obtained for the 30 minutes adsorption of FGN onto SiO_2 surfaces coated with PLL/HEP multilayers measured by means of QCM-D. The panel (a) corresponds to the interaction with the PLL-ended coatings and (b) with the HEP-ended coatings. Experiments run at 25 °C and pH 7.4 for a protein concentration of 0.2 mg/ml in DPBS

3.3 Summary of the results

Each of the proteins used, BSA, IgG and FGN shows a different behavior depending on the surface the protein solution is exposed to:

a) When the surface is negatively charged, as it is the case of bare SiO_2 and HEP-ended coatings, BSA is unable to adsorb onto it. However, when the surface is positively charged, the case of PLL-ended multilayers, serum albumin forms a monolayer and gets conformationally changed due to the strong electrostatic interactions.

b) IgG binds to both SiO_2 and PLL-ended coatings although as the number of layers increases the mass and the thickness of the protein layer decreases, suggesting that the conformation that the antibody adopts onto the coating is influenced by presence of PLL and the number of layers. On the other hand, similarly to BSA, IgG is unable to adsorb onto HEP-ended coatings.

c) FGN is able to interact with all the used surfaces showing the highest adsorption for the PLL-ended coatings and the lowest for HEP coatings. Furthermore, as the number of layers increases FGN loses its ability to bind onto HEP as a result of the increasing content of HEP that would result in a stronger electrostatic and steric repulsion forces.

Studying the adsorption of blood proteins onto the surfaces of vascular medical devices, especially fibrinogen, is an indirect manner to verify their hemocompatibility [18] as the formation of proteinaceous layers onto the blood/device interface can lead to clot formation [3] and the malfunction of such a device [4]. According to the results obtained in this study

we have found that for when model SiO_2 surfaces are coated with three PLL/HEP bilayers, blood protein fouling almost vanishes compared to uncoated surfaces or PLL-ended coatings.

4. Conclusions

We have found that blood protein adsorption onto poly-L-lysine/heparin multilayers depends on both the number of layers and the polyelectrolyte present on the outermost layer. While the three proteins used in this study adsorb onto PLL-ended coatings, protein adsorption is almost negligible when heparin is on the topmost layer for coatings formed by three PLL/HEP bilayers. These results indicate that this coating system could be used to prevent blood protein adsorption when heparin is present on the outermost layer. As this is a key factor for the development of hemocompatible coatings, we propose that multilayers composed by PLL and HEP could be employed as low fouling coatings for vascular medical devices such as dialysis tubing and membranes.

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